

CLAIMS:

1. A method of assaying for folate in a folate containing sample, wherein at least some of said folate comprises at least one attached glutamate residue, said method comprising:

subjecting said sample to hydrolysis to release paraaminobenzoic acid, p-aminobenzoyl glutamic acid, or a salt thereof; contacting the released paraaminobenzoic acid, p-aminobenzoyl glutamic acid, salt, or a diazo derivative thereof, with a binding partner therefor; and directly or indirectly detecting the resulting binding partner:paraaminobenzoic acid, binding partner:p-aminobenzoyl glutamic acid, or salt or derivative combination.

2. A method as claimed in claim 1 wherein said method does not comprise any chromatographic separation steps.

3. A method as claimed in claim 1 or claim 2 wherein said sample is a blood derived sample.

4. A method as claimed in any of claims 1 to 3 wherein said binding partner is selected from an antibody, an antibody fragment, a single chain antibody, a single chain antibody fragment, an oligopeptide, an oligonucleotide and a small organic molecule.

5. A method as claimed in claim 5 wherein said small organic molecule is an aromatic tertiary amine, phenol or phenol derivative capable of forming a diazo compound with paradiazobenzoic acid (PDBA) or paradiazobenzoyl glutamate (PDBA-glu).

6. A method as claimed in any of claims 1 to 5 wherein said hydrolysis comprises treating said sample with a metal catalyst under acidic conditions.

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7. A method as claimed in any of claims 1 to 6 wherein said hydrolysis comprises treating said sample with microwave radiation.

8. A method as claimed in any of claims 1 to 7 wherein said hydrolysis comprises treatment with an oxidising agent.

9. A method as claimed in claim 8 wherein the oxidising agent is hydrogen peroxide and/or potassium permanganate.

10. A method as claimed in any of claims 1 to 9 wherein said hydrolysis comprises treatment with a reducing agent.

11. A method as claimed in claim 10 wherein the reducing agent is sodium borohydride.

12. A method as claimed in any of claims 1 to 11 wherein said hydrolysis comprises oxidative photolysis.

13. A method as claimed in claim 12 wherein said oxidative photolysis is carried out in the presence of a photosensitiser.

14. A method as claimed in any of claims 1 to 13 wherein said sample is incubated in the presence of naturally occurring and/or added enzymes whereby to remove all but the terminal glutamate residue from said folate and wherein the product of the hydrolysis is PABA-glu.

15. A method as claimed in any of claims 1 to 13 wherein said sample is incubated in the presence of at least one added enzyme, whereby to remove all glutamate residues from said folate, and wherein the product of

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the hydrolysis is PABA.

16. A method as claimed in any of claims 1 to 15 wherein said binding partner: paraaminobenzoic acid, binding partner: p-aminobenzoyl glutamic acid, or salt or derivative combination is detected directly by absorbance or fluorescence.

17. A method as claimed in any of claims 1 to 15 wherein said binding partner: paraaminobenzoic acid, binding partner: p-aminobenzoyl glutamic acid, or salt or derivative combination is detected indirectly by means of a secondary binding partner.

18. A kit for use in the performance of the assay of the invention, said kit comprising:

- i) a folate hydrolysis reagent; and
- ii) a PABA, PABA-glu, PDBA or PDBA-glu binding partner;

19. A kit as claimed in claim 18 additionally comprising an enzyme or enzyme cocktail.

20. A kit as claimed in claim 18 or claim 19 additionally comprising a PABA to PDBA or PABA-glu to PDBA-glu converting reagent.

21. A kit as claimed in any of claims 18 to 20 additionally comprising a secondary binding partner.

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